Electronic Supplementary Material (ESI) for Chemical Communications.

Supporting Information

Chiral rhodium(III)-azobenzene complexes as photoswitchable DNA molecular locks

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Experimental Section

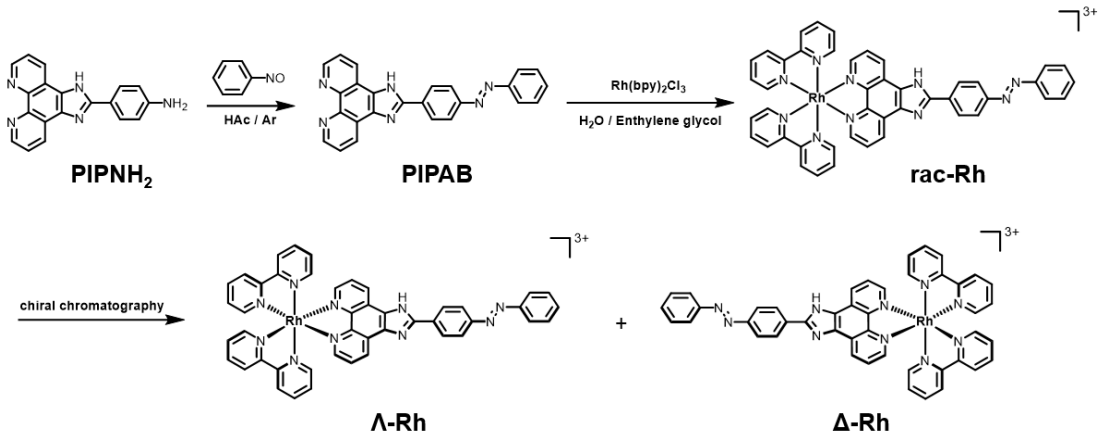
Supporting figures and Tables

Materials and instruments

Unless otherwise noted, all chemical reagents and solvents were commercially available and used without further purification. Double distilled (DD) water was used throughout all of the experiments. 1,10-Phenanthroline-5,6-dione, 4-Nitrobenzalde, and nitrosobenzene were purchased from Energy Chemical. NaCl, KCl, EDTA, HEPES, and ethidium bromide (EB) were obtained from Sangon Biotech. DNA sequences, Premix TaqTM, DL5000 DNA Marker, TaKaRa MiniBEST Agarose Gel DNA, and Extraction Kit Ver.4.0 were purchased from Takara (Japan). Microanalysis (C, H, and N) was carried out using a Perkin-Elmer 240Q elemental analyzer. The ^{1}H and proton-decoupled ^{13}C NMR spectra were recorded on a Bruker advance III Nuclear Magnetic Resonance Spectrometry. High-resolution electrospray ionization mass spectra (HR-ESI-MS) were recorded by Orbitrap LC/MS (Q Exactive, Thermo, German). The UV-Vis spectra were recorded on a Varian Cary 300 spectrophotometer. Emission spectra were recorded on a PerkinElmer LS 55 fluorescence spectrometer at room temperature. CD spectra were recorded on a Jasco J-810 spectropolarimeter in a 1 cm path length cylindrical quartz cell at room temperature. AFM images were obtained on a Nanoscope 8.15 multimode scanning probe workstation using nonconductive silicon nitride nanoprobes (probe model NP-S10, Bruker) under ambient conditions in the ScanAsyst mode. The photoisomerization of the azobenzene moiety in Δ/Λ-Rh was accomplished by irradiating with light from a desktop UV analysis (365nm, 48 W) and a Xenon lamp through an appropriate filter (480 nm, 300 W).

Synthesis and characterization

$[Rh(bpy)_2Cl_2]Cl{\cdot}H_2O$[1] and $PIPNH_2$[2] were prepared according to the previous literatures. Synthetic routes of **Δ-Rh** and **Λ-Rh** were described in **Scheme S1**.



Scheme S1 Synthetic routes of **Δ-Rh** and **Λ-Rh**.

Synthesis of PIPAB

1.24 g $PIPNH_2$ (4.0 mmol) was dissolved in 10 mL acetic acid and allowed to add 0.48 g nitrosobenzene (4.5 mmol) before removing air by argon for 30 minutes. The reaction mixture was stirred at room temperature overnight, after which 20 mL water was added and aqueous sodium carbonate was used to neutralize the solution. Then, the product was extracted by chloroform (3 × 20 mL) and the organic layer was collected, dried over anhydrous sodium sulfate, and evaporated to afford the crude product, which was further purified by silica gel column chromatography using chloroform/methanol (10:1, v/v) as eluent to obtain orange solid, 0.48 g. Yield: 30 %. ^{1}H NMR (300 MHz, DMSO-d_6) δ 14.01 (s, 1H), 9.06 (d, J = 3.9 Hz, 2H), 8.97 (d, J = 8.0 Hz, 2H), 8.52 (d, J = 8.5 Hz, 2H), 8.15 (d, J = 8.5 Hz, 2H), 7.96 (d, J = 6.6 Hz, 2H), 7.87 (s, 2H), 7.63 (d, J = 7.0 Hz, 3H). Proton-decoupled ^{13}C NMR (126 MHz, DMSO-d_6) δ 152.53, 149.94, 148.50, 148.38, 144.26, 132.91, 132.23, 130.17, 130.02, 129.41, 127.97, 127.63, 127.19, 123.85, 123.13, 121.22, 120.50. HR-ESI-MS: *m/z:* cald. for $[C_{25}H_{17}N_6]^+$([M+H]$^+$) = 401.15092;

found,.401.14964.

Synthesis of $[Rh(bpy)_2(PIPAB)](PF_6)_3$ (*rac*-Rh)

$[Rh(bpy)_2Cl_2]$ Cl (0.056 g, 0.1 mmol) and $PIPNH_2$ (0.040 g, 0.1 mmol) were dissolved in 10 mL ethylene glycol and heated to reflux at 135 °C under argon atmosphere in the dark. After reaction for 12 hours, the mixture was cooled to room temperature and diluted with 30 mL water. Afterwards, saturated KPF_6 aqueous solutions were added to afford great amounts of precipitation, which was subsequently filtered, washed with water and diethyl ether and dried over *vacuum*. The crude product was purified by ultra-high-pressure liquid chromatography using water and methanol (70/30 - 70/30 - 0/100, v/v) as eluent. Yield: 70%. ^{1}H NMR (600 MHz, Acetonitrile-d_3) δ 9.40 (d, *J* = 8.3 Hz, 2H), 8.76 (d, *J* = 8.2 Hz, 2H), 8.72 (d, *J* = 8.1 Hz, 2H), 8.58 (d, *J* = 8.1 Hz, 2H), 8.53 (t, *J* = 8.0 Hz, 2H), 8.41 (t, *J* = 7.9 Hz, 2H), 8.11 (d, *J* = 8.1 Hz, 2H), 8.02 – 7.97 (m, 4H), 7.92 (d, *J* = 5.3 Hz, 2H), 7.85 (d, *J* = 5.5 Hz, 2H), 7.82 – 7.78 (m, 2H), 7.64 (t, *J* = 7.3 Hz, 2H), 7.61 (d, *J* = 5.5 Hz, 3H), 7.57 (t, *J* = 6.7 Hz, 2H). Proton-decoupled ^{13}C NMR (101 MHz, Acetonitrile-d_3) δ 157.04, 155.04, 154.84, 152.98, 152.66, 151.28, 151.23, 148.49, 143.36, 143.19, 142.17, 135.82, 134.27, 131.61, 130.57, 130.50, 129.45, 127.76, 127.52, 126.89, 126.78, 126.47, 123.36, 122.74. HR-ESI-MS: *m/z:* cald. for $[C_{45}H_{31}N_{10}Rh]^{2+}$($[M-3PF_6-H]^{2+}$) = 407.08886; found, 407.08759.

Separation of Enantiomers

In order to obtain two isomers from ***rac*-Rh**, high-performance liquid chromatography (HPLC) equipped with chiral column (CYCLOBOND I 2000 DMP) was used with a gradient eluent from KPF_6 (20 mM, aq.)/acetonitrile/ethanol (40/10/50, v/v/v) to KPF_6 (20 mM, aq.)/acetonitrile/ethanol (40/50/10, v/v/v). **Δ-Rh** was firstly collected and then **Λ-Rh** was obtained separately. Identified by circular dichroism (CD) spectrum, the solution with identical optical rotation was collected and loaded for further resolution until the CD signals remain highest. Then, the solvent was evaporated and dried over a *vacuum* to afford two isomers in 10 % yield.

Δ-Rh: ^{1}H NMR (300 MHz, Methanol-d_4) δ 9.54 (d, *J* = 9.3 Hz, 2H), 8.99 - 8.92(m, 4H), 8.62 - 8.54 (m, 4H), 8.47 (t, *J* = 7.7 Hz, 2H), 8.10 (d, *J* = 8.2 Hz, 2H), 8.05 – 7.96 (m, 7H), 7.90 -7.79 (m, 3H), 7.74 (d, *J* = 5.5 Hz, 2H), 7.66 – 7.53(m, 5H). CD [λ(Δε), H_2O): 362 (-0.4), 320 (-6.6), 294 (2.5 M^{-1} cm^{-1}).

Λ-Rh: ^{1}H NMR (300 MHz, Methanol-d_4) δ 9.57 – 9.48 (m, 2H), 8.98 – 8.91 (m, 4H), 8.63 – 8.52 (m, 4H), 8.46 (t, *J* = 7.9 Hz, 2H), 8.10 (d, *J* = 8.4 Hz, 2H), 8.02 – 7.96 (m, 8H), 7.88 (t, *J* = 6.7 Hz, 2H), 7.74 (d, *J* = 5.7 Hz, 2H), 7.67 – 7.51 (m, 5H). CD [λ(Δε), H_2O): 362 (0.5), 322 (6.4), 293 (-4.7 M^{-1} cm^{-1}).

Photoisomerization of Δ/Λ-Rh using UV-Vis spectra

Δ/Λ-Rh solutions were prepared in Tris 1 buffer with a concentration of 5 μM. For trans-to-cis transformation, the solutions were subjected to irradiation (365 nm) and recorded by UV-Vis spectroscopy with an interval of 1 second. For *trans*-to-*cis* process, the irradiation source was shifted to visible light (480 nm) for 15 sec.

Stabilities of *cis*-forms of Δ/Λ-Rh using UV-Vis spectra

Stabilities of *cis*-form of **Δ/Λ-Rh** were identified by UV-Vis spectra. When spectra of **Δ/Λ-Rh** reached to plateaus upon irradiation (365 nm), indicating the complete transformation of *cis*-form. Then, the stabilities of such form in 37 °C were monitored by UV-Vis spectra at 385 nm in long time scales with an interval of 30 minutes.

HPLC-MS trace of Δ/Λ-Rh photoisomerization process

Δ/Λ-Rh samples in different photostationary state (dark, 365 nm, 480 nm) with a concentration of 10 μM (0.1 % methanol) in aqueous solution were subjected to HPLC-MS assay. Detailed elution condition was listed in the Table S3.

Photoisomerization of Δ-Rh using ^{1}H NMR spectroscopy

10 mg of **Δ-Rh** was dissolved in acetonitrile-d_3 for ^{1}H NMR spectroscopy assay. Afterwards, solution in NMR tube was transferred to a cuvette and subjected to irradiation (365 nm, 15 sec).

Transcription inhibition *in vitro*

Transcription inhibition *in vitro* was performed according to the previous literature[3]. Template DNA (*In vitro* Transcription T7 Kit, Takara) is capable of transcription into two different length RNA with T7 RNA polymerase. Template DNA was incubated in RNase Free dH_2O with different concentrations of Rh(III) complexes in 37 °C for 15 sec. Subsequently, groups of dark were kept in the dark while groups of light were illuminated with UV light (365 nm) for 15 sec. Then, the mixture was added by T7 RNA Polymerase, 10 × Transcription Buffer, ATP, GTP, CTP, and UTP to afford the final volume of 20 μL. After reaction for 2 hours at 42 °C, 2 μL bromophenol blue was added to quench the reaction and serve as the electrophoretic indicator. Samples were then loaded to agarose gel (1%) electrophoresis using a constant voltage power supply (90 V) for an hour. Agarose gels were stained with EB solutions and imaged with Tanon 1600 Automatic imaging analysis system. Protocol of groups of pre-365 nm were identical to the aforementioned methods except that Rh(III) complexes were exposed to illumination (365 nm, 15 sec) prior to be added into other transcription substrates.

2-kb Linear DNA preparation for AFM study

2-kb Linear DNA were prepared according to our previous work[4]. The 2-kb DNA fragment was generated by PCR using pBR 322 plasmid DNA as a template. Then, the 2-kb DNA fragment was isolated from the reaction buffer by 1% (w/v) electrophoresis gel. Electrophoresis was carried out in TBE (111 mM Tris-HCl, 111 mM Boric acid, 4 mM EDTA, pH 8.3) as the running buffer for 2 h at 80 V and at room temperature. Finally, PCR assays were purified using a TaKaRa MiniBEST Agarose Gel DNA Extraction Kit from agarose gel. The concentration of the 2 kbp DNA fragment was determined by a micro-ultraviolet spectrophotometer (MAESTRO).

AFM samples preparation and imaging

Samples of 2-kb linear DNA (8 ng mL^{-1}) were prepared and incubated with **Δ-Rh** (5 μM) or **Λ-Rh** (5 μM) in buffer solutions(3 mM $MgCl_2$, 4 mM HEPES, 5 mM KCl, pH 7.2) and the final volume was adjusted to 50 mL. For dark groups, samples were treated with or without helicase and keep in dark environment. For light groups, after treating with and without helicase, samples were illuminated with UV light (365 nm) for 15 sec. Following digestion, each sample was loaded to a mica surface (2 cm × 2.5 cm) and evaporated to dryness. The remaining salts were rinsed with ultrapure water (200 mL) for three times and then dried with Argon flow. The images were captured in 256 × 256 pixels format and analyzed with the software accompanying the imaging module. Protocol of groups of pre-365 nm were identical to the aforementioned methods except that Rh(III) complexes were exposed to illumination (365 nm, 15 sec) prior to be incubation with 2-kb linear DNA.

DNA photocleavage experiments

For the gel electrophoresis experiment, supercoiled pBR322 DNA (10 ng/μL) was treated with the Rh(II) complex in Tris-2 buffer. The soln. was then stored in the dark or irradiated at r.t. with a desktop UV analysis. After incubation, the reaction mixture was quenched by the addition of 2 μL gel loading dye (0.25% bromophenol blue, 0.25% xylene cyanol, 40% glycerol, and 2 mM EDTA). The samples were subjected to electrophoresis at 80 V on 0.8% agarose gel in TBE buffer (pH 8.3). The gel was stained with 1 μg/mL ethidium bromide solution, visualized

with UV light, and photographed for analysis using a Tanon 1600 Gel Imaging System.

Thermal denaturation studies

Thermal denaturation studies were carried out with a Perkin-Elmer Lambda 35 spectrophotometer equipped with a Peltier temperature-controlling programmer (± 0.1 °C). The absorbance at 260 nm was continuously monitored for solutions of 10 bq DNA (5'-CGTTAGTTCA-3'/3'-GCAATCAAGT-5', 1 μM) in the absence and presence of the **Δ/Λ-Rh** (5 μM) and with or without illumination (365 nm, 15 sec). The temperature of the solution was increased by 1.0 C min^{-1}. Protocol of groups of pre-365 nm were identical to the aforementioned methods except that Rh(III) complexes were exposed to illumination (365 nm, 15sec) prior to be incubation with 10 bq DNA.

MALDI-TOF-MS spectroscopy

10 bq DNA (5'-CGTTAGTTCA-3'/3'-GCAATCAAGT-5', 5 μM) in Tris 1 buffer was used for stability of conjugation of DNA and **Δ/Λ-Rh** (100 μM). DNA was mixed with **Δ/Λ-Rh** and kept in 4 C overnight. Afterwards, samples were irradiated at 365 nm (15 sec) for light group.

Competitive binding studies

CT-DNA (100 μM) and ethidium bromide (EB, 10 μM) were mixed in 9 mL buffer solution and kept in 4 C overnight. 3 mL of mixture were transferred to quartz cuvette. Stock solutions of **Δ/Λ-Rh** (10 mM) were added to the cuvette in a portion (0.3 μL) and allowed to bind with DNA for 3 min. Afterwards, emissions of EB were recorded ranging from 500 – 700 nm, excited by 340 nm. Quenching constant (*K*) was calculated by the classical Stern–Volmer equation[5]:

$I_0/I = 1 + Kr$

Where I_0 and *I* are the emission in the absence and the presence of complex, respectively. *K* is a linear Stern–Volmer quenching constant dependent on the ratio of r_{be}(the ratio of the bound concentration of EB to the concentration DNA). *r* is the radio of the total concentration of complex to that of DNA.

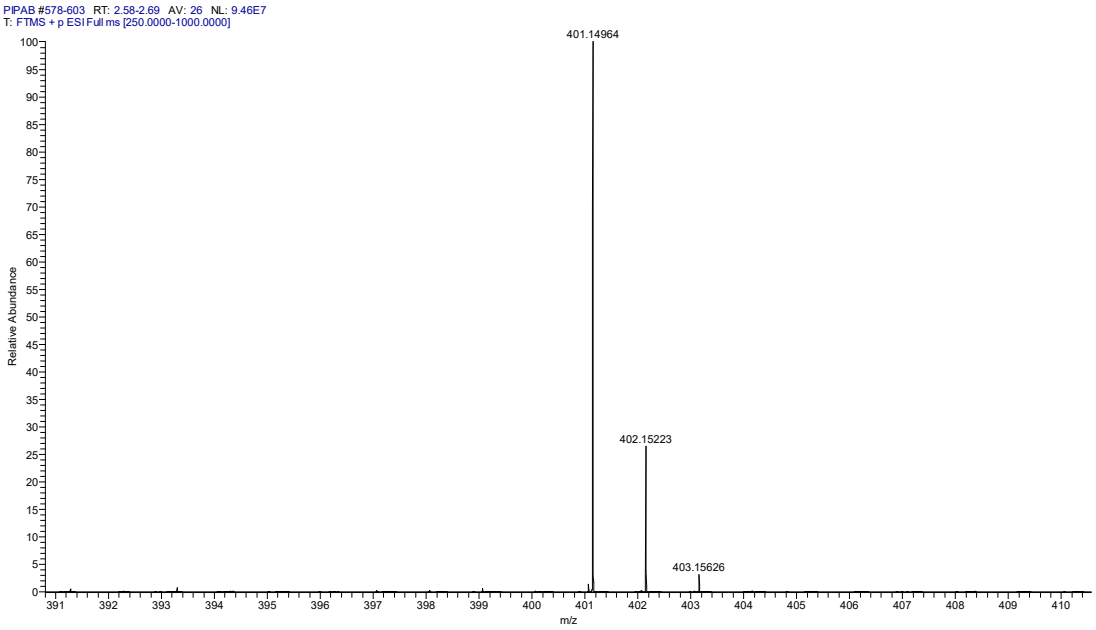


Fig.S1 HR-MS spectrum of **PIPAB**.

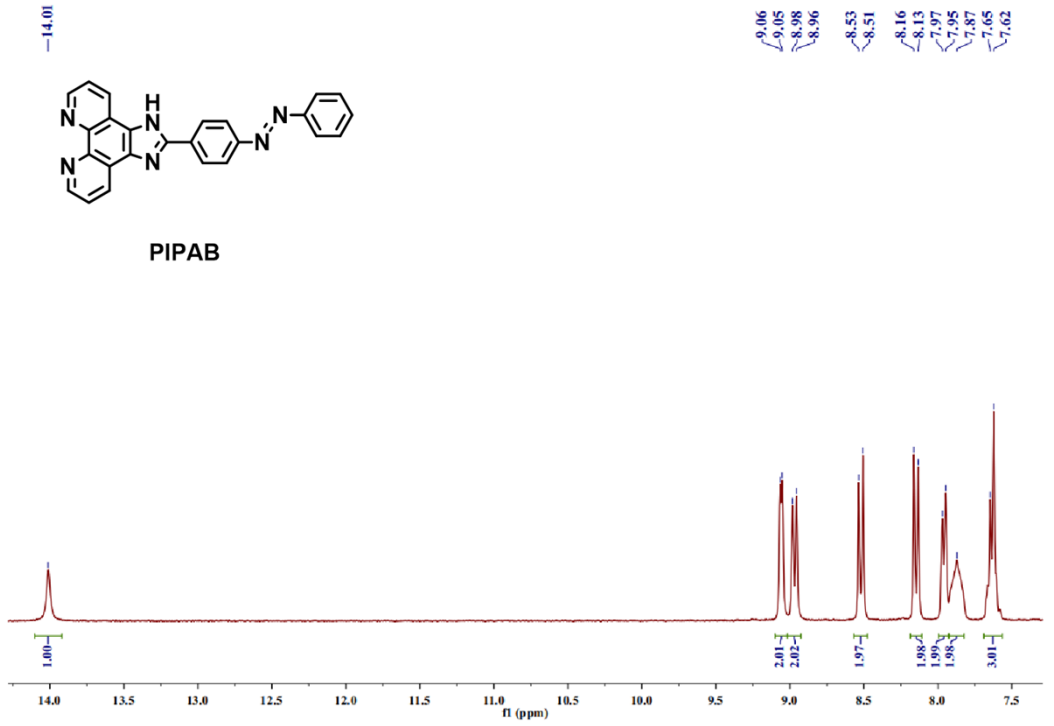


Fig.S2 ^{1}H NMR spectrum of **PIPAB**.

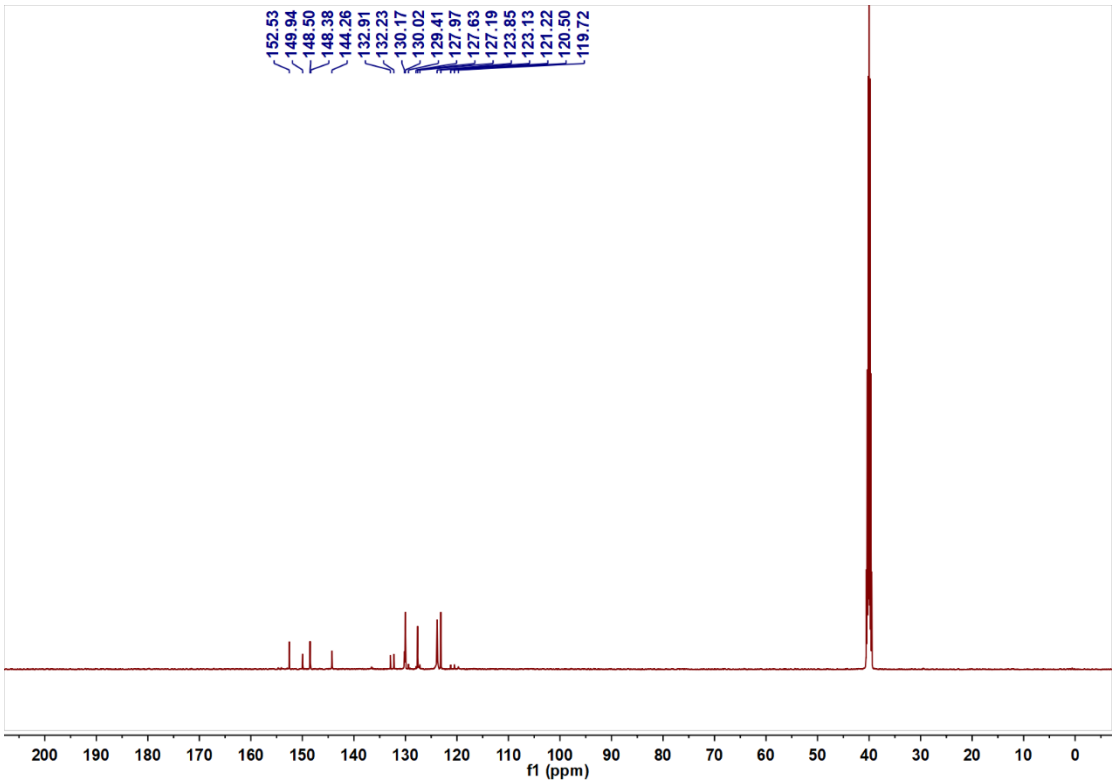


Fig. S3 Proton-decoupled ^{13}C NMR spectra of **PIPAB**.

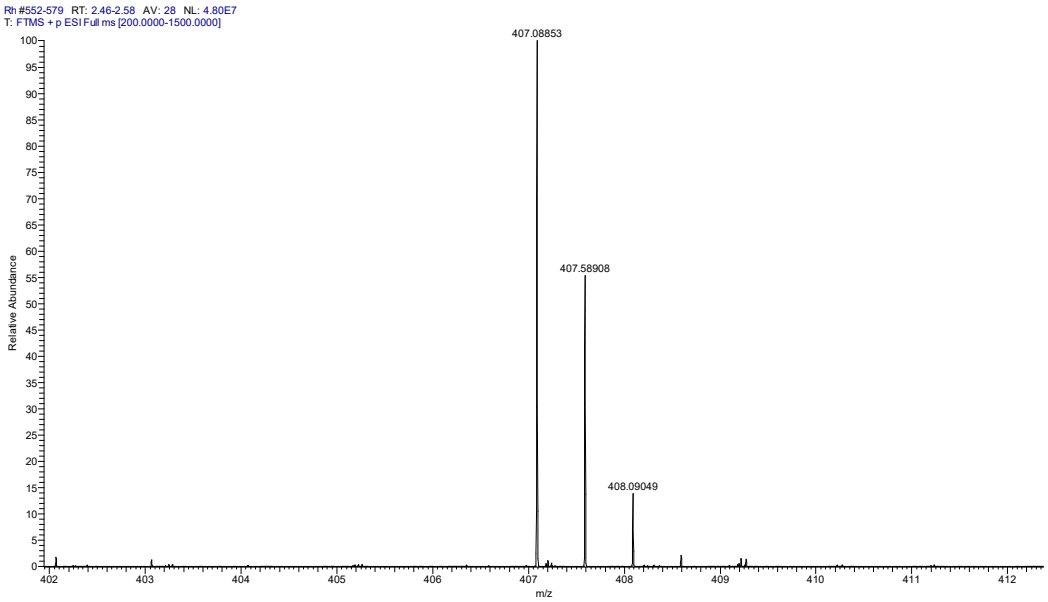


Fig.S4 HR-MS spectrum of ***rac*-Rh**.

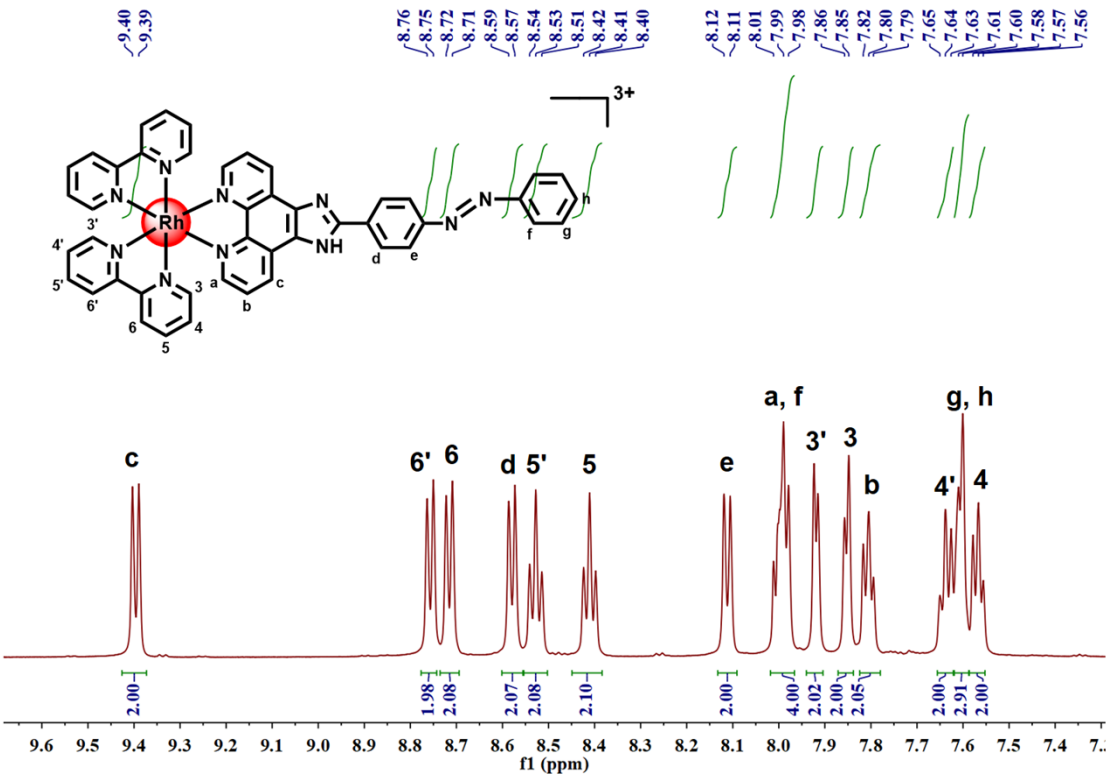


Fig.S5 ^{1}H NMR spectrum of ***rac*-Rh.**

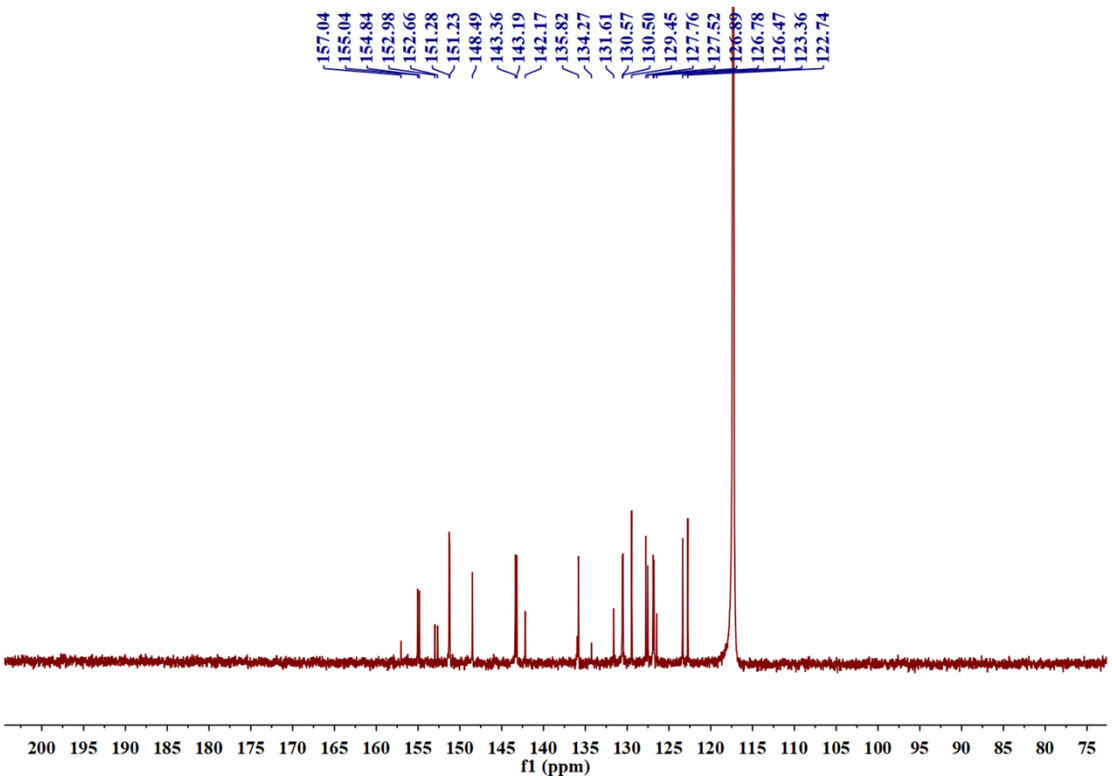


Fig. S6 Proton-decoupled ^{13}C NMR spectra of ***rac*-Rh**.

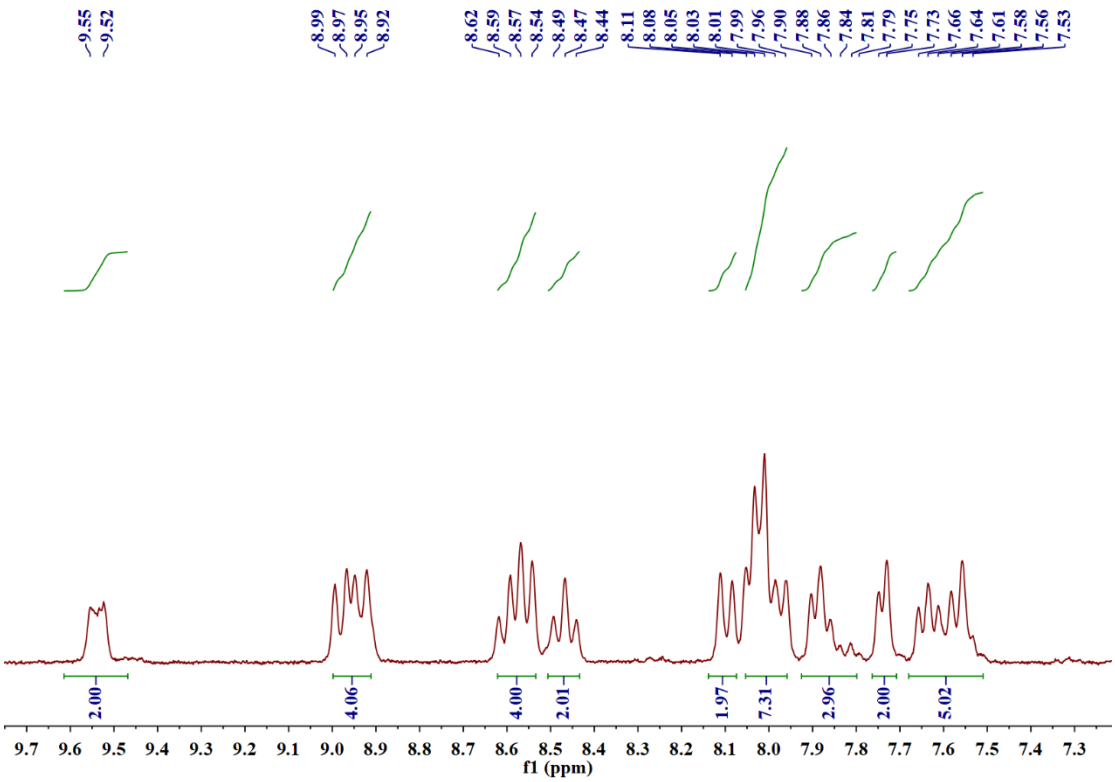


Fig. S7 ^{1}H NMR spectra of **Δ-Rh**.

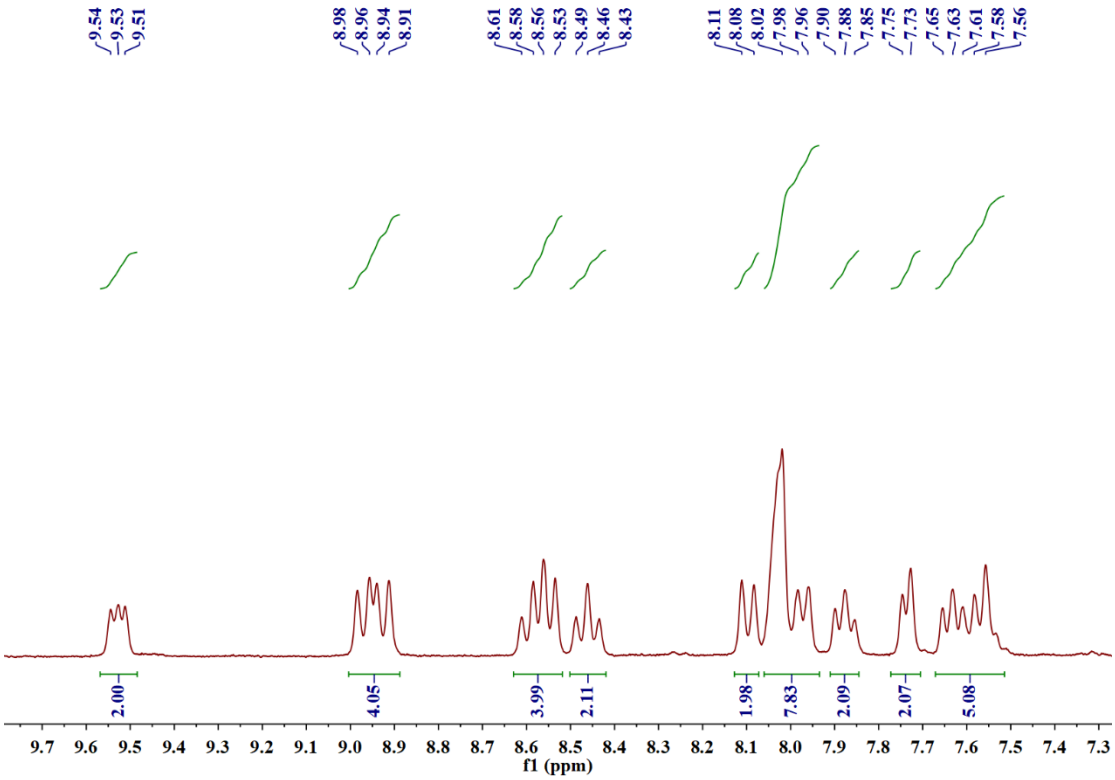


Fig. S8 ^{1}H NMR spectrum of **Λ-Rh.**

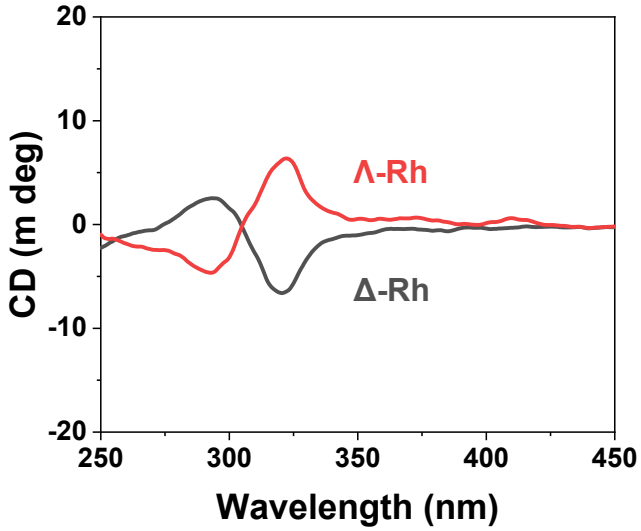


Fig. S9 CD spectra of **Δ-Rh** and **Λ-Rh**.

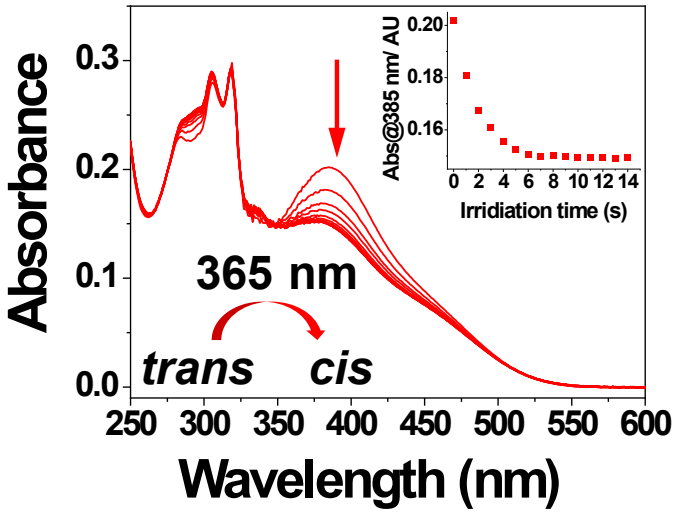


Fig. S10 *Trans-cis* photoisomerization of **Λ-Rh** upon light irradiation (365 nm). Inset: Change of absorption at 385 nm in dependence of the irradiation time.

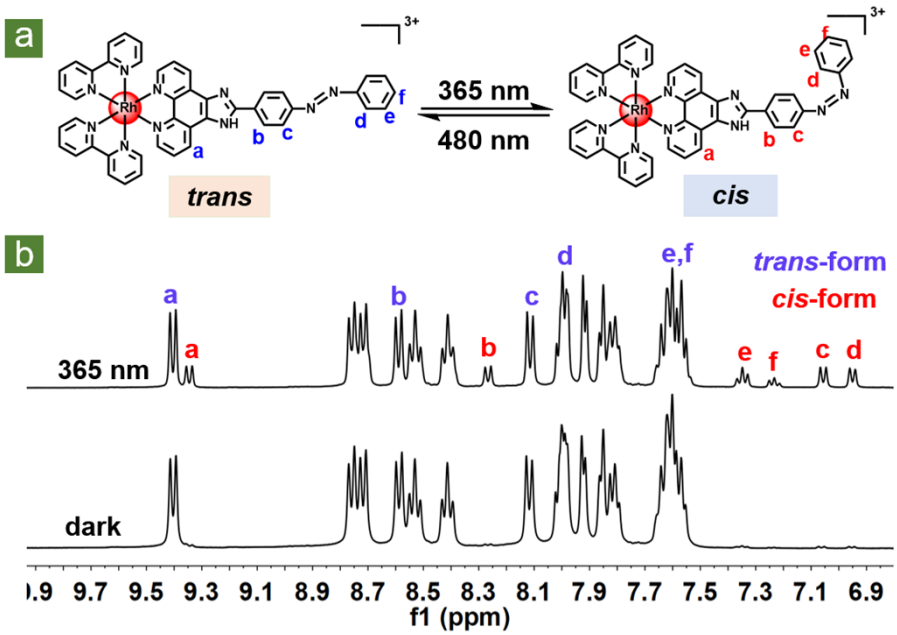


Fig. S11 (a) Photoisomerization of **Δ-Rh**; (b) ^{1}H-NMR spectra of **Δ-Rh** in the dark and upon irradiation at 365 nm.

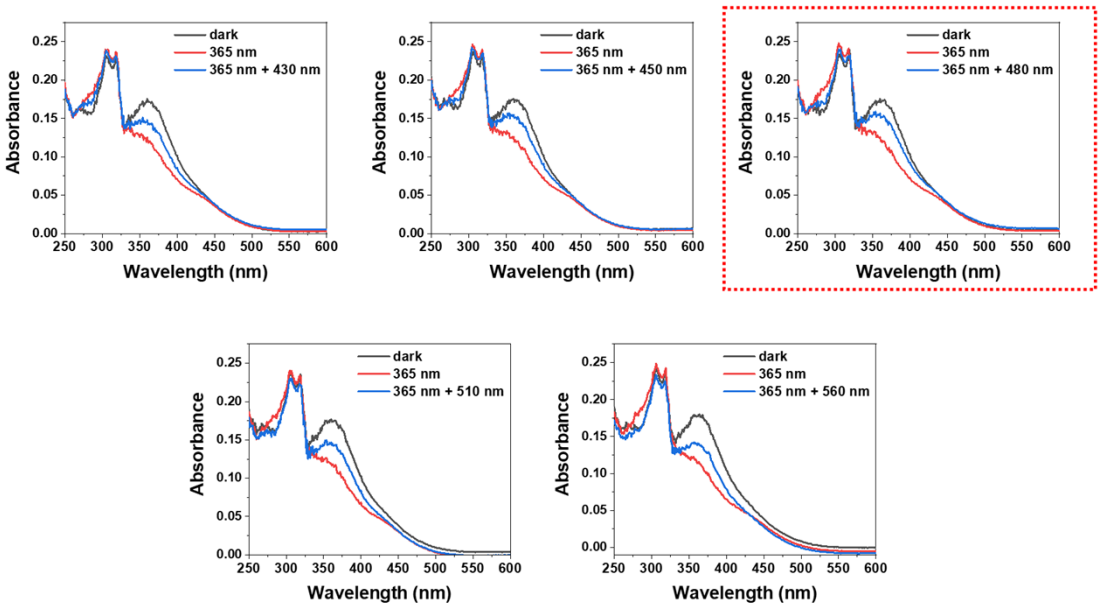


Fig. S12 *Cis-trans* photoisomerization of **Δ-Rh** upon irradiation at various wavelengths.

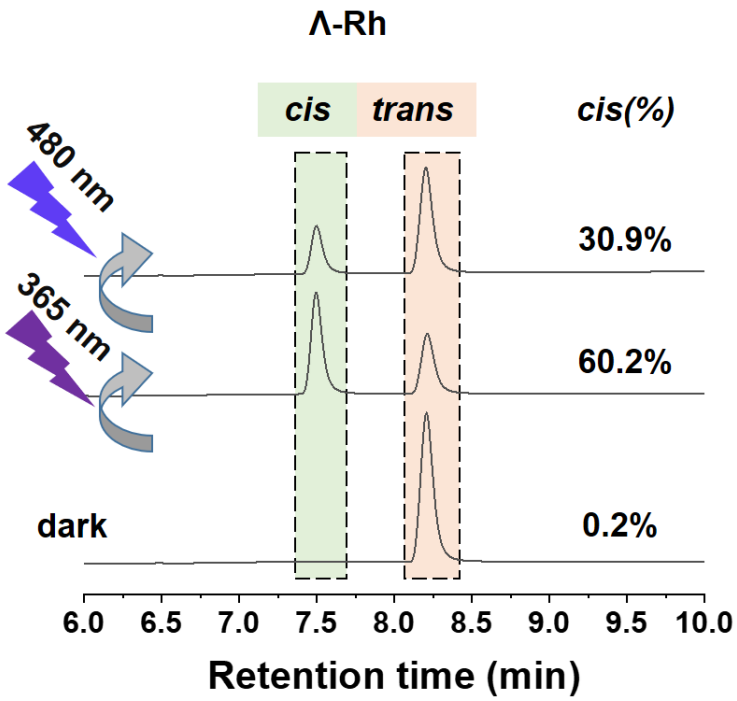


Fig. S13 HPLC-MS trace of *trans-cis* photoisomerization.

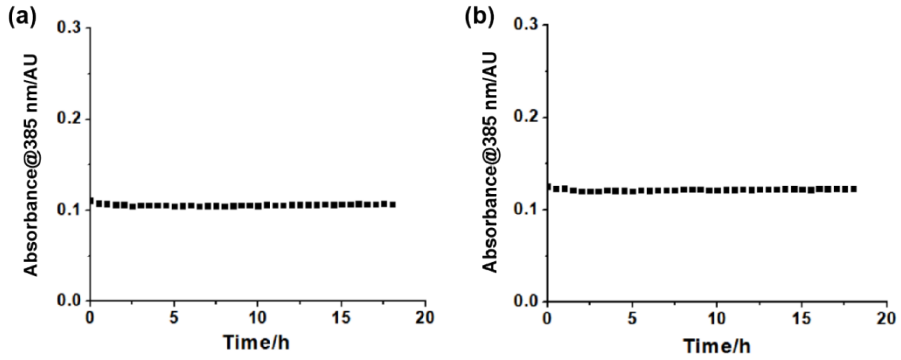


Fig. S14 Stability of the *cis*-form of **Δ-Rh** (a) and **Λ-Rh** (b) monitored by UV-Vis absorption spectroscopy (385 nm, 37 °C).

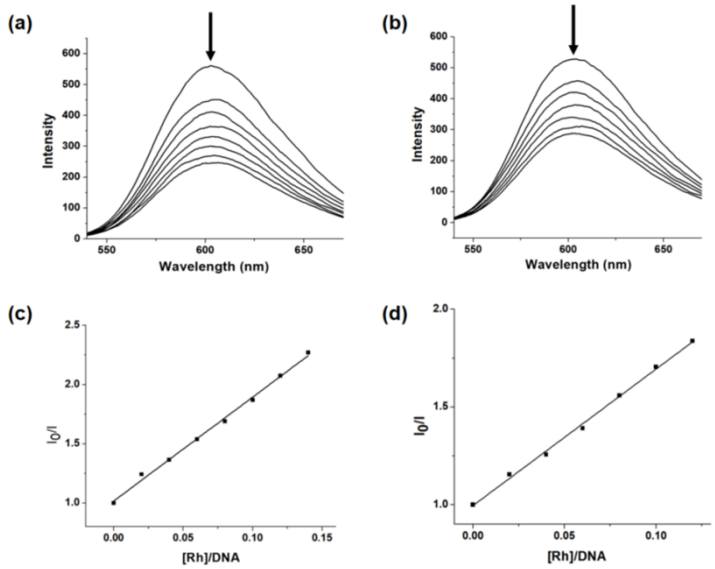


Fig. S15 Emission spectra of ethidium bromide intercalated CT-DNA ([ethidium bromide] = 4 μM, [CT-DNA] = 80 μM) upon addition of increasing concentration of **Δ-Rh** (a) or **Λ-Rh** (b). Plots of I_0/I of [**Δ-Rh**]/[DNA] (c) or [**Λ-Rh**]/[DNA] (d).

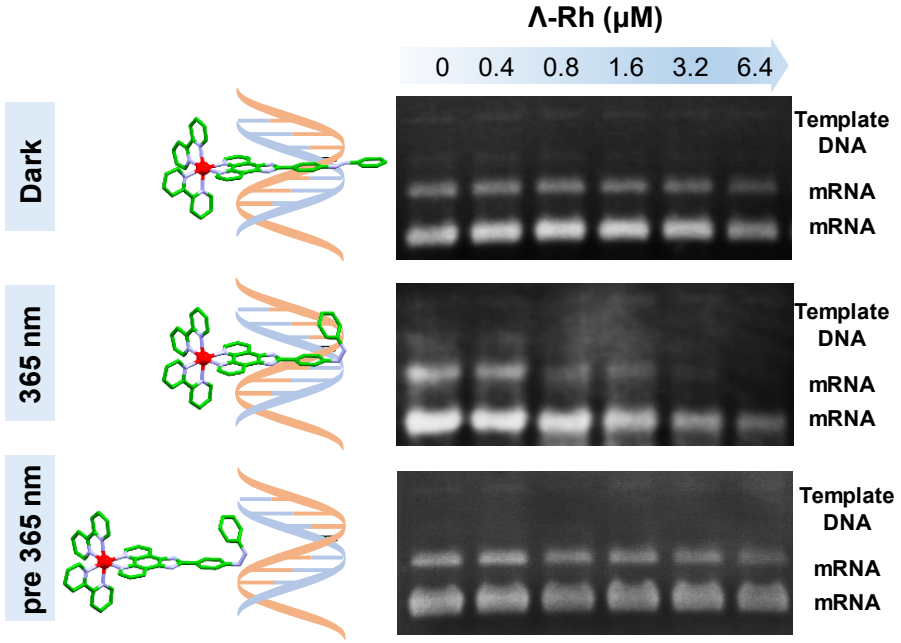


Fig. 16 Inhibition of the RNA replication by **Λ-Rh** upon incubation with the DNA in the dark (dark), incubation with the DNA in the dark followed by irradiation (365 nm), or irradiation followed by incubation with the DNA in the dark (pre 365 nm).

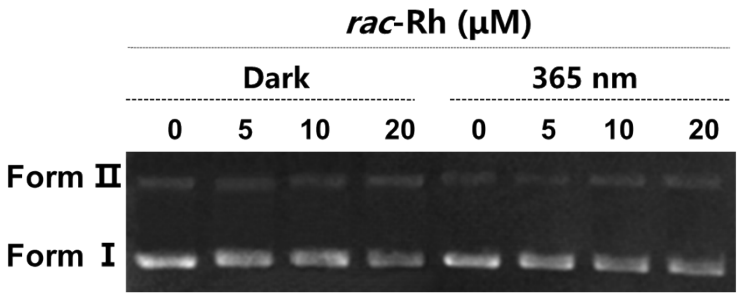


Figure S17 Agarose gel electrophoresis pattern of the supercoiled pBR322 DNA (10 ng/μL) in the dark or under 365 nm irradiation with or without the Rh(III) complexes for 15 sec. Lane 1, DNA alone in the dark; lane 2-4, DNA + ***rac*-Rh** (5 to 20 μM) in the dark; lane 5, DNA alone upon irradiation at 365 nm; lane 6-8, DNA + ***rac*-Rh** (5 to 20 μM) upon irradiation at 365 nm.

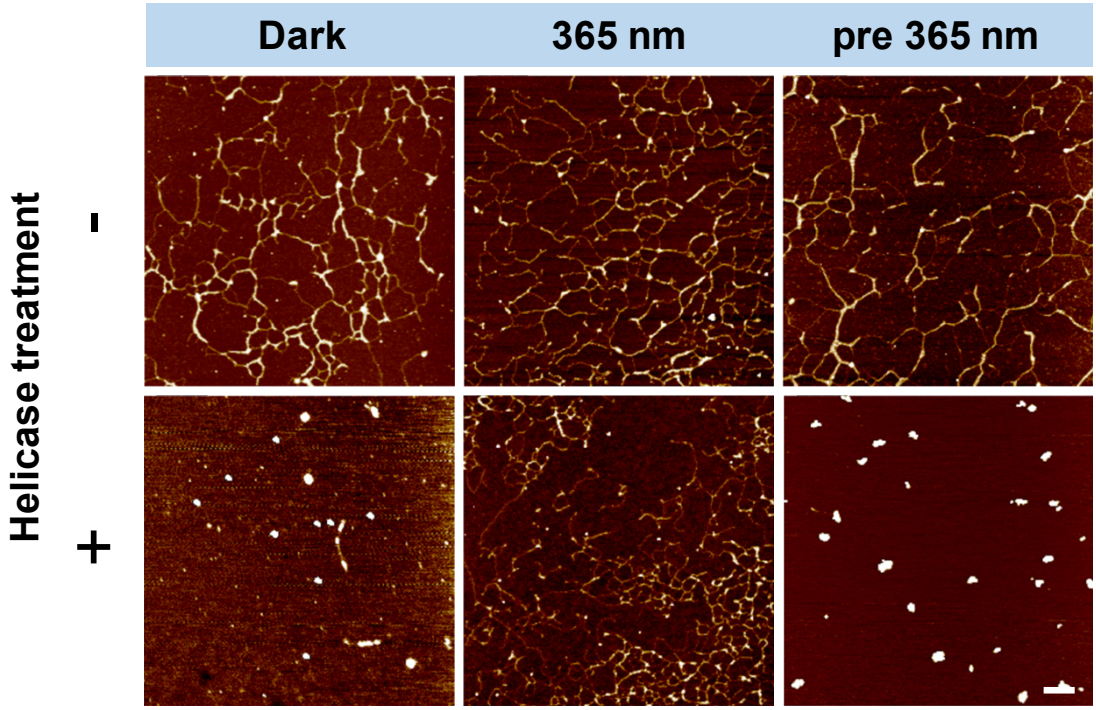


Fig. S18 Atomic force microscopy image of the morphology of linear DNA with and without helicase treatment and upon incubation of **Λ-Rh** with the DNA in the dark (dark), incubation of **Λ-Rh** with the DNA in the dark followed by irradiation (365 nm), or irradiation followed by incubation of **Λ-Rh** with the DNA in the dark (pre 365 nm). Scale bar: 200 nm.

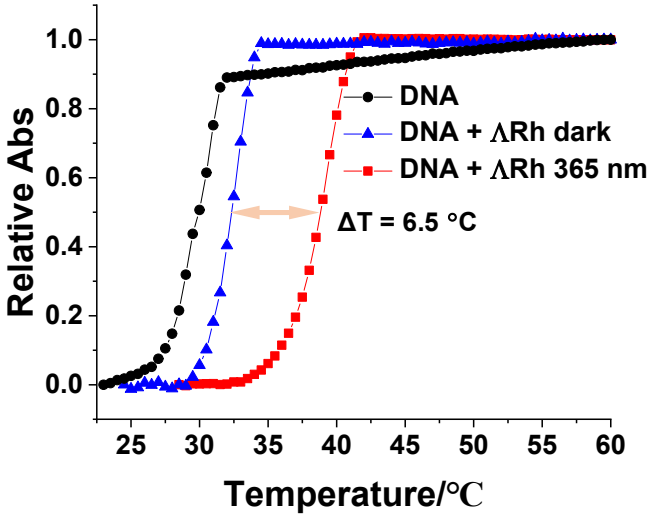


Fig. S19 Melting temperature curve of DNA double helix (black) incubated with **Λ-Rh** in the dark (blue) and upon 365 nm irradiation (red).

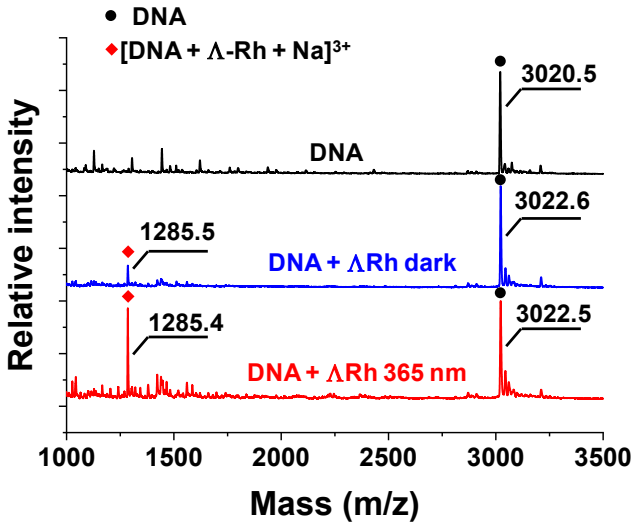
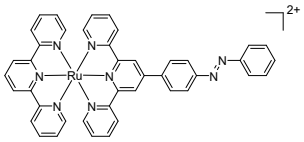
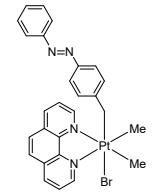
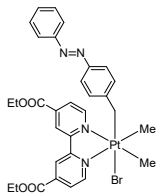
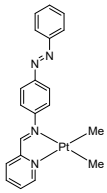
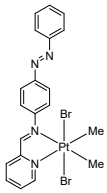
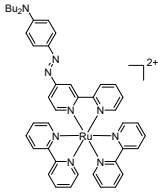


Fig. S20 MALDI-TOF MS of DNA (black), the incubation of DNA with **Λ-Rh** in the dark (blue) or upon irradiation at 365 nm (red).

Table S1 Photoisomerization properties of **Δ/Λ-Rh**.

Compounds	$\Phi_{t\rightarrow c}$	***% cis*** **(365 nm)**	***% cis*** **(480 nm)**
Δ-Rh	0.467	69.3	34.3
Λ-Rh	0.555	60.2	30.9

Table S2 Photoisomerization efficiencies of reported metal complexes appended with azobenzene group.

Complexes	Photoisomerization efficiencies (%)	References
	17.4	*Inorg. Chem.* 2001, **40**, 4986-4995
	60	*Organometallics* 2013, **32**, 2552-2557
	75	*Organometallics* 2013, **32**, 2552-2557
	25	*Organometallics* 2012, **31**, 6262-6
	40	*Organometallics* 2012, **31**, 6262-6269
	0	*Chem. - Eur. J.* 2015, **21**, 8262 – 8270

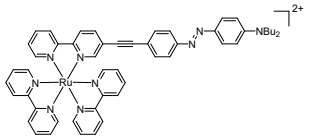
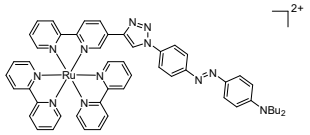
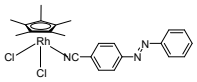
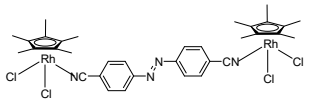
	28 (THF), 0 (DCM)	*Chem. - Eur. J.* 2015, **21**, 8262 – 8270
	25 (THF), 15 (DCM)	*Chem. - Eur. J.* 2015, **21**, 8262 – 8270
	19	*J. Organomet. Chem.*2001, **640**, 10 – 20
	47	*J. Organomet. Chem.*2001, **640**, 10 – 20

Table S3 Melting temperature (T_m/˚C) of the 10bp duplex DNA with or without **Δ/Λ-Rh** under different conditions (in the dark or under 365 nm irradiation).

	T_m/°C		
	dark	**365 nm**	**ΔT_m**
10bp DNA	30.1	30.6	0.5
10bp DNA **+Δ-Rh**	31.7	43.4	11.7
10bp DNA **+Λ-Rh**	32.4	38.9	6.5

Table S4 detailed conditions of HPLC-MS.

Retention(min.)	A(%)	B(%)	Flow(mL/min.)
0.00	70	30	0.5
10.00	0	100	
Chromatographic column: Shim-pack GIST C18; Dim.(mm):250*4.6; Particle Sz.(u): 5			
Injection volume: 4 µL			
Multi-Step Gradient: A(H_2O, 0.1 % TFA), B(MeCN)			

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